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The development of body and organ shape

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Abstract

Background: Organisms show an incredibly diverse array of body and organ shapes that are both unique to their taxon and important for adapting to their environment. Achieving these specific shapes involves coordinating the many processes that transform single cells into complex organs, and regulating their growth so that they can function within a fully-formed body.

Main text: Conceptually, body and organ shape can be separated in two categories, although in practice these categories need not be mutually exclusive. Body shape results from the extent to which organs, or parts of organs, grow relative to each other. The patterns of relative organ size are characterized using allometry. Organ shape, on the other hand, is defined as the geometric features of an organ's component parts excluding its size. Characterization of organ shape is frequently described by the relative position of homologous features, known as landmarks, distributed throughout the organ. These descriptions fall into the domain of geometric morphometrics.

Conclusion: In this review, we discuss the methods of characterizing body and organ shape, the developmental programs thought to underlie each, highlight when and how the mechanisms regulating body and organ shape might overlap, and provide our perspective on future avenues of research.

Keywords: Body shape, Organ shape, Allometry, Geometric morphometrics, Morphogens, Environmentally-sensitive growth, Organ patterning

Background

Whether it was intended, when Charles Darwin stated that "endless forms most beautiful and most wonderful have been, and are being, evolved" he elegantly captured how much of the diversity we observe across organisms arises because they differ in shape [1] (Fig. 1). This simple observation has inspired over a hundred years of research into how shape changes between populations, species, and taxa. More recently, investigators have begun to probe the genetic mechanisms that give rise to body and organ shape [2–4]. This is, of course, not a simple task as the genetic pathways directing how shape develops are varied and complex, and may not be conserved across organisms. Even so, by comparing across organisms we could potentially identify common properties between the cellular and genetic pathways that build body shape.

One way to begin identifying common properties involves defining what we mean by body shape. The definitions of body shape can be sorted, rather broadly, into two overlapping categories [5]. The first characterizes body shape by the relative size of their component parts (Fig. 1). The patterns described by changes in relative size between organs are known to specialists in this field as allometry [6, 7]. Allometric patterns characterize not only the size of organs relative to each other, but how changes in the size of one organ scales with another [8] (Fig. 2a). As such, measures of allometry provide a method for characterizing whole body shape.

Practitioners differ in how they define allometry, and these differences are divided into two main schools of thought. The Huxley-Jolicoeur school describes allometry as variation among traits resulting from differences in their size [6, 9]. In contrast, the Gould-Mosimann

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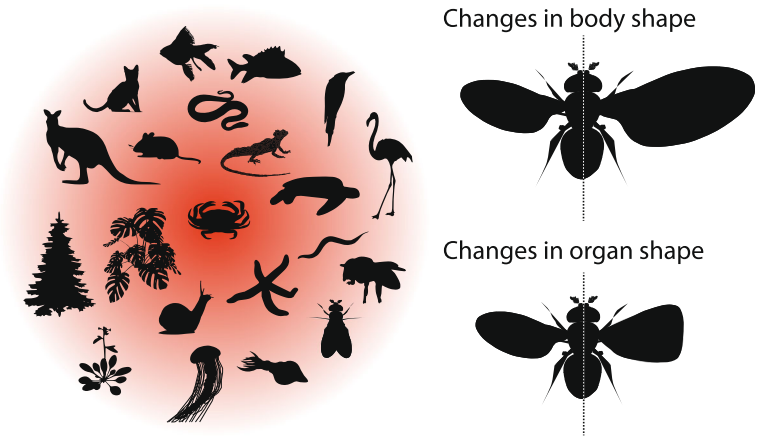

Fig. 1 Much of the morphological diversity seen in multicellular organisms results from changes in body and organ shape. Animals, plants, and other multicellular organisms differ in their body forms. This diversity of body shapes is generated by modifying body shape – or the relative size of organs compared to the rest of the body, or by changing organ shape – the three-dimensional configuration of an organ's features independent of its size

school conceptually distinguishes shape from size and measures the difference in shape as the variation of proportions independent of size [5, 7, 10]. This review will define allometry in terms of how traits differ in their relative size (Huxley-Jolicoeur definition). However, we will return to how organ shape varies with organ size when discussing how and when the mechanisms regulating each might overlap.

The second way of thinking about body shape is to consider all geometric properties of a body part, but to exclude its size [11, 12]. The geometric properties of organ shape are commonly described using the relative position of morphological features that can be readily identified across specimens, known as landmarks, while accounting for size, orientation, and position (Fig. 1, [13]). Describing the geometry of organs in this way – known as geometric morphometrics – provides a sophisticated measure of organ shape (Fig. 2b).

Countless examples describe how organ shape changes as organs increase in size [14–16], demonstrating that shape and size are likely to share developmental regulators. Studies over the past twenty years, primarily from insects, have highlighted key genetic pathways required for regulating body size and relative organ size [2, 4, 17–20]. These studies have provided new insight into the molecular mechanisms that underlie the differences in growth across organs that are responsible for generating allometric patterns. In parallel, studies in plants and animals have begun to determine the molecular mechanisms resulting in organ shape [21–25]. In this review, we will first outline the different methods used to characterize body and organ shape, before delving into

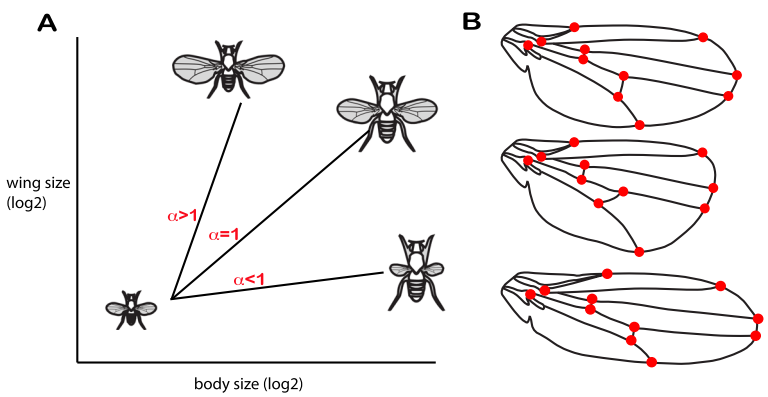

Fig. 2 Tools for quantifying organ shape using allometry (**a**) or geometric morphometrics (**b**). **a** shows the scaling relationships between mean trait size (wing size) and body size. Traits are isometric when $\alpha = 1$. Hypoallometry describes a relationship where $\alpha < 1$ and trait y shows little or no change in size with increasing body size. For hyperallometric relationships ($\alpha > 1$), trait y increases disproportionately with increasing body size. **b** shows how the shape of the wing can be measured using the spatial relationship of discrete landmark points (shown in red). The position of these cartesian coordinates can be altered to produce new wing shapes independent of wing size

the recent literature that describes genetic pathways regulating each. Finally, we will highlight evidence that shows the extent to which the mechanisms that give rise to body and organ shape overlap, with views to future avenues of research. While most of our examples arise through insights from the widely studied *Drosophila melanogaster*, we also provide examples from other animals, as well as from plants.

Main text

Quantifying variation in body and organ shape

A number of excellent reviews describe and compare the methodologies used to study body shape, including metrics for describing allometric patterns, and organ shape, the realm of geometric morphometrics [5, 26–28]. It is not our intention to provide an exhaustive review of these methods, but rather to highlight the central concepts pertaining to describing patterns of allometry and of organ geometry, and to identify where these differ. We will use this comparison later in the review to outline the mechanisms known to regulate body shape and organ shape.

Body shape – patterns of allometry

In studies of allometry, shape is characterized by measuring how the dimensions of two body parts scale with one another [8]. Typically, these relationships are measured using size variables such as width, length, area, or volume. This allows one to compare how organ size scales with body size, or how the size of specific regions within an organ correlates with the size of the whole organ. Allometry can be used to characterize the variation in scaling either across developmental time (ontogenetic allometry), across individuals of the same species at the same developmental stage (static allometry), or across species of the same developmental stage (evolutionary allometry) [7, 29, 30].

Allometry is modelled mathematically using Huxley's allometric equation, which describes the relationship between two traits, for example between trait "y" (e.g. brain size) and trait "x" (e.g. head perimeter), as $y = \beta x^{\alpha}$. Log-transforming the allometric equation linearizes this relationship such that the log of trait y is a function of the log (β) plus the log of trait x times the constant α [8]. β will therefore be the value when log(x) = zero, or when x = 1, and corresponds to the elevation of the allometric relationship [31].

$$\log(y) = \log(\beta) + \log(x)$$

The allometric elevation, indicated as β, provides information about mean trait size. The constant α is known as the allometric coefficient (or scaling exponent), describes the slope of the line or the rate of change in one trait (y) relative to change in another (x) (Fig. 2a) [3, 6].

Depending on the value of the allometric coefficient α, scaling relationships are categorized into three types. Isometry, where α = 1, occurs when both traits scale proportionally (Fig. 2a). This type of relationship occurs between the maxillary palps and thorax area of *Drosophila melanogaster* [32]. Increasing the slope away from isometry would result in a hyperallometric relationship (α > 1), where trait y becomes disproportionately larger in response to an increase in trait x (Fig. 2a). A classic example, the scaling relationship between major claw size and body size in male fiddler crabs is hyperallometric [6, 33]. Hypoallometry (α < 1) occurs when the size of trait y increases slowly with increasing size for trait x (Fig. 2a). This hypoallometric relationship is typical of the genital structures in male insects, which vary little in size as male body size increases [4, 32, 34, 35].

Patterns of allometry can also be used to compare how scaling relationships change with genotype, environmental conditions, and sex, as well as across developmental trajectories. When measured across environmental conditions, the allometric coefficient reflects a trait's sensitivity to those conditions – otherwise known as plasticity [36]. For example, when *D. melanogaster* larvae are reared across a range of nutritional conditions, body size in the emerging adults increases with increasing diet quality. Wing size in the adults scales isometrically with body size, whereas the size of the genital arch shows a hypoallometric relationship with body size [32]. Thus, the wing shows higher nutritional plasticity than the genital structures. In cases where traits vary discretely in size across environmental conditions, such as in polyphenisms, the allometric elevation can be used to describe trait plasticity. For example, the mean size of the eyespots on the wings of *Bicyclus anynana* butterflies is significantly larger when animals are reared at wet season temperatures (27 °C), than when they are reared at the lower dry season temperature of 19 °C [37]. These differences in how organs respond to environmental change have recently been mined to uncover developmental genetic mechanisms underpinning the development of allometric relationships [2, 4, 38].

Within a species, because allometric relationships are typically measured on populations of genetically diverse individuals, genetic differences in scaling relationships can easily be missed. Determining how scaling relationships vary between individuals is difficult since each individual can only present one size phenotype [4, 39, 40]. Nevertheless, theoretical models highlight how the distribution of individual scaling relationships in a population can greatly affect how allometry evolves [40]. Using clonal species or isogenic lines, where all individuals in a line are essentially genetically identical, provides a tool to explore the

effects of genetic variation on scaling relationships [4, 40]. These studies promise to generate important insight into the sources of genetic variation that cause scaling relationships to vary within a population. Perhaps more importantly, they open up new opportunities to develop an understanding of how allometric relationships evolve [4, 40].

Organ shape – describing organ geometry

Allometric patterns can also be used to describe organ shape by comparing the relative size of one organ compartment against another. At a tissue or even cellular level, the relationship between measures that differ in dimensions can provide useful shape information. For example, when cells or organs grow isomorphically, i.e. do not alter their shape during growth, the allometric coefficient between log mass and log length is equal to 3. Allometric coefficients < 3 indicate that the cell or organ is flattening as it increases in size, whereas coefficients > 3 show that the cell or organ is increasing in thickness with size [41]. Such relationships provide a reasonable estimate of changes in organ shape.

Geometric morphometrics offers a more precise map of organ shape that can be analysed both independently from organ size and in the context of other non-shape variables [42]. In this sense, shape is described as all geometric features of an organ excluding size, position, and orientation [5, 27]. This approach considers the magnitude and location of morphological variation [43], providing a more complete picture of the specific features of an organ that give rise to changes in organ shape.

Geometric morphometrics has been widely applied across organisms and fields, and is divided into either landmark-based morphometrics or outline-based morphometrics. In landmark-based morphometrics, shape is summarized in terms of the spatial relationship of discrete landmark points of correspondence (anatomical loci), which are either described as 2- or 3-dimensional Cartesian coordinates [13, 42, 44] (Fig. 2b). Outline-based morphometrics involves summarizing the shape of open or closed perimeters with the use of semi-landmarks [42, 45]. Semi-landmarks describe contours or boundary outlines and do not depend on the presence of true anatomical landmarks [45]. Both types of methodology provide precise descriptions of organ shape independent from organ size.

While both landmark and outline-based approaches describe organ shape, they differ in terms of what can be inferred from the data. In landmark-based approaches, each landmark point is a formal hypothesis that assumes that corresponding landmarks across individuals are homologous [46]. This allows the explicit testing of how the distribution of these homologous structures varies across development or within a population, or how it evolves between species [46, 47]. For example, landmark techniques have been applied in identifying hybrids between species and subspecies of the western honey bee [48, 49], and in understanding how human facial features differ in terms of their perceived masculinity [50]. Landmark-based methods can nevertheless be problematic when novel structures arise, as there will be no corresponding homologues in species that do not share the novelty. In contrast, outline-based morphometrics do not assume homology between the parts of organs [51]. Although this frees shape analysis from the confines of landmarks, when comparing across development, individuals in a population, or species, one cannot ascribe changes to specific physical structures using outline-based approaches [45].

To be able to separate organ size from organ shape, researchers using landmark-based approaches scale landmarks to the same centroid size using Generalized Procrustes Analysis [27, 52, 53]. Centroid size is the square root of the summed squared distances between all landmarks and the centroid (the average x and y coordinates, and in 3D, z coordinates across all landmarks) [5]. Generalized Procrustes Analysis translates all landmarks between two objects to the same position and orientation, and then transposes and translates them to a common centroid size [5, 46]. This Procrustes superimposition describes shape differences as the variance between the landmark configurations that cannot be removed by scaling, translating, or rotating landmark points [5]. Transforming data in this way allows organ shapes to be compared across individuals, populations, or species, but also allows the relationship between organ size and organ shape to be assessed.

Regardless of the approach, geometric morphometrics allows researchers to address questions about how variation in the shape of biological structures can be coordinated at developmental, functional, and/or evolutionary levels. When features that contribute to shape co-vary, this is known as morphological integration [47, 54]. Integration generally implies that the features contributing to shape share a developmental regulator, a similar function, or a common genetic or evolutionary origin [54, 55]. In contrast, when shapes of organs show distinct spatial or temporal patterns of variation, this is known as modularity [47, 56]. Modularity can imply that the developmental mechanisms that underpin the generation of each module is distinct [56, 57]. Organs can show both morphological integration and modularity. For example, in insect wings the landmarks obtained from the wing veins show greater co-variation within either the anterior or posterior compartment than between compartments [54, 58–60]. Similarly, facial patterns and outlines show greater co-variation within a sex than between sexes in humans [50, 61, 62]. Across

development, there is a high degree of co-variation in the relative timing of development of the components of the oro-nasal region relative to the cranium in mammals and lizards [56, 57]. In this way, the spatial and temporal patterns of variation in organ shape can indicate whether developmental regulators should be expected to be the same or to differ across component parts.

Regulating body and organ shape

The processes that give rise to body shape and those that generate organ shape could, in principle, be controlled by different developmental mechanisms. The scaling relationships that give rise to body shape result from organ growth, which is regulated by both organ-specific (or organ autonomous) growth signals and systemic signals [63]. Organ shape, on the other hand, results from the mechanisms that establish cell identities and dictate cell behaviours, otherwise known as patterning. These patterning mechanisms delineate similarities and differences between cell types, map the positions of specific structures, and also control organ-autonomous growth.

However, when we consider the numerous studies showing how organ shape varies across organ sizes it seems that the mechanisms defining organ shape and size are likely to overlap [5, 32, 54]. For example, during development as the body grows the increasing need for blood flow necessitates an increase in the size and an accompanying change in shape of the human heart [33, 64]. Below we discuss the developmental mechanisms that regulate body and organ shape, highlight where these mechanisms might intersect.

Developmental mechanisms of allometry

The final size of all organisms is determined by both the rate of growth and the duration of the growth period [2]. Developmental mechanisms that control the rates of growth and duration of growth have been studied most extensively in insects, and are regulated by two broadly classified mechanisms: organ-autonomous growth, and environmentally-sensitive, or plastic growth [65]. Where organ-autonomous growth involves internal developmental programs that ensure that an organ grows sufficiently to function properly, plastic growth matches the organism's size to its environment. These two developmental programs work together by integrating the signals received from either organ-autonomous regulators or systemic mechanisms [65, 66].

Organ-autonomous control of growth Organs are known to have characteristic, autonomous sizes that arise due to the developmental mechanisms that specify, pattern, and regulate organ growth. Evidence for this autonomous property of organ size was first provided by studies of *D. melanogaster*. In this species, wings and other adult structures develop as pouches of cells – known as imaginal discs – within the growing larvae [67]. When wing imaginal discs are transplanted from early stage larvae into the abdomen of an adult, these discs grow to be the same size as a normal late-stage wing disc and then stop growing [68]. This is not a phenomenon unique to insects. In humans when a lobe of liver is transplanted into a recipient both the donor and original liver grow to near-normal sizes [69]. These studies demonstrate that organs have autonomous properties that ensure that they grow to the correct size.

For many organs and in many different developmental contexts, gradients of morphogens determine organ-autonomous size. The term morphogen, as originally defined by Turing [70], is a chemical substance (generally a protein) that is unevenly distributed across a field of cells such that it forms a gradient by diffusion. This gradient is interpreted by the receiving cells and used to generate distinct cell identities and behaviours. Morphogens in several contexts are scale invariant; they produce the same patterning outcomes regardless of the size of the field of cells on which they act. For example, removal of 30% of the cells in a blastula-stage zebrafish embryo leads to smaller yet perfectly proportioned embryos [71]. In these embryos proportional scaling occurs because of changes in the concentration of two interacting morphogens: Nodal and Lefty. When cells are removed, the concentration of the highly diffusible morphogen Lefty increases, and acts to inhibit Nodal expression thereby rescaling the whole embryo to the correct dimensions [71]. Similarly, in the wing imaginal disc of *D. melanogaster* morphogens like Decapentalegic (Dpp) and Wingless (Wg) regulate the size of the wing in a scale-invariant manner [72–74]. Manipulating the size of the posterior compartment of the wing disc causes rescaling of the Dpp morphogen gradient to the appropriate proportions [74]. In addition to their roles in regulating organ-autonomous growth, the gradients of Dpp and Wg establish the anterior/posterior and dorsal/ventral axis of the wing disc [75]. Thus, morphogens and their gradients play a key role in determining the overall shape of organs, as well as the scaling relationships within an organ and between the organ and the whole body.

Differences in morphogen activity among organs can play an important role in relative organ size. At the end of development, the wing discs in *D. melanogaster* are approximately 3.5–4-fold bigger than the disc that gives rise to the balancing organ, the haltere [76]. These differences are regulated by interactions between morphogens and the Hox gene Ultrabithorax (Ubx), responsible for providing segment-specific identities to the organs of the third thoracic segment and abdominal segments.

Ubx acts to reduce the concentration of Dpp, as well as limiting its spread and activity in the haltere relative to the wing [76–78]. In this way, modulating morphogens in an organ-specific manner is a key factor in regulating the elevation (the β term) of the allometric equation between the size of the haltere relative to the size of the wing. Taken together, morphogens appear to regulate the organ-autonomous properties of growth while patterning genes that confer segment-specific identity tune the properties of these morphogens so that the organ is of the appropriate scale. With new technologies that allow levels of endogenous protein expression in live cells [79, 80], we can now begin to explore how properties of morphogen gradients differ across organs to result in organ-specific sizes.

Mechanical properties of tissues are also thought to affect relative size. The ventral nerve cord of developing *D. melanogaster* embryos is 60% the length of the embryo regardless of embryo size. The ventral nerve cord depends on interactions between the ventral nerve cord cells and the extracellular matrix to achieve this scaling [81, 82], potentially due to tension created between the two. In the wing disc, cells at the edges of the disc cease dividing towards the end of development [83]. The mechanical strain that this imposes on the more central dividing cells is thought to shut down their cell division, thereby controlling the final size of the wing disc [73]. Pathways like the Hippo and JNK pathways both regulate organ growth and are sensitive to mechanical stress [84, 85], thus these pathways are proposed to be central for regulating organ-autonomous size.

Finally, while morphogen gradients and mechanical stress regulate the way that many organs grow, organs whose size is dictated by cell migration, such as organs that grow via branched tubular networks, require different types of cues to know when to stop growing. For example, the size and shape of the hermaphroditic gonad in *Caenorhabditis elegans* depends on the migration of the distal tip cells [86]. These distal tip cells migrate along the ventral surface before turning dorsally and migrating along the dorsal surface to form a U-shaped structure [86]. In mutants for the transcription factor Pax6 (vab-3), the distal tip cells continue migrating forming large, mishapened gonads [87]. Pax6 regulates gonad size by controlling the expression of two α integins, Pat-2 and Ina-1. Integrins are transmembrane receptors that facilitate interactions with the extracellular matrix, and are thus important for pathfinding during cell migration in many contexts [88]. In the gonad, Pat-2 is turned on by Pax6, and seems to be necessary for correct pathfinding in the distal tip cells, as reducing its expression causes ventralised distal tip cell migration or extra turns in the gonad [87]. In contrast, Ina-1 is downregulated by Pax6. Failure to turn off Ina-1 results in perpetually growing gonads [87]. Integrins also play central roles in tubular growth directed by tip cells in many other animals, such as the renal tubes of *D. melanogaster* [89], vertebrate angiogenesis [90, 91], and the development of the branched respiratory systems in mammals and insects [92, 93]. How this mechanism of growth regulation scales across organ and body sizes is poorly understood.

Environmentally-sensitive organ growth While organs have their own autonomous sizes, body and organ growth is also sensitive to a wide range of environmental conditions. The signalling pathways that respond to most environmental conditions have yet to be resolved, however we have a solid understanding of how animals respond to nutrition during development to regulate their growth. In *D. melanogaster*, nutrition is sensed by an endocrine organ known as the fat body [94–97]. The fat body detects the availability of dietary nutrients and communicates nutritional status to the brain via a number of secreted peptide hormones [98–104]. These peptides regulate the production and secretion of insulin-like peptides by specialized insulin producing cells in the brain, which in turn modulate growth and maintain nutritional homeostasis.

The *D. melanogaster* genome encodes a family of seven insulin-like peptides (dILPs 1–7) and a relaxin-like peptide (dILP8) [105, 106]. While dILPS 1–7 are secreted by distinct cells in the different tissues of the body [107–109], dILP8 is secreted by damaged imaginal discs [110, 111]. dILPs alter body size in response to nutrition [66]. During feeding, increased nutrients induce the synthesis and secretion of dILPs 2, 3 and 5 from a group of neurosecretory cells in the brain called the insulin-producing cells (IPCs) [106, 112]. While the specific peptides might not be conserved, animals as distantly related as insects, nematodes, and vertebrates use insulins and /or insulin-like growth factors to tune their growth to environmental conditions [66, 113, 114]. These circulating insulins bind to and activate insulin receptors (InR in *D. melanogaster*) on target peripheral organs, activating a conserved phosphorylation cascade that induces growth via the protein kinase Akt [115–118]. In this way, insulin signalling acts systemically to link nutrition to body and organ growth.

A second pathway regulates growth in response to nutrition in a cell-autonomous manner - the highly conserved target of rapamycin (TOR) pathway. TOR was first discovered in yeast as the target of the growth inhibitory drug Rapamycin [119], but has since been found in all eukaryotes [120]. In yeast and animal cells, TOR kinase occurs in two distinct multi-protein complexes, TORC1 and TORC2, with different cellular functions which both contribute to growth and viability. TORC1 is

sensitive to Rapamycin and under high amino acid concentrations is activated via Ras Homolog Enhanced in Brain (Rheb) [121]. This pathway controls growth in animal cells through the S6 Kinase 1 (S6K1) and the initiation factor 4E-binding protein 1 (4E-BP1) S6K [122]. TORC2 is rapamycin insensitive but controls the full activation of the protein kinase Akt to mediate growth [123, 124]. TOR signaling initiates translation and ribosome biogenesis, stimulates rRNA synthesis, and promotes cell autonomous growth [123]. Loss of TOR signaling causes developmental and growth arrest and reduces nuclear size of cells, a phenotype typical of animals under amino acid starvation [20]. Because the insulin and TOR pathways share several downstream regulators, including Akt, they are often referred to as the insulin/TOR pathway [125].

All organs respond to insulin/TOR signaling to regulate their growth, however the sensitivity of organs to this signaling differs [126]. While the wings of many insects show isometric scaling with body size, the genital disc scales hypoallometrically [32]. In *D. melanogaster* and the dung beetle *Onthophagus nigriventris,* differences in scaling between these organs are underpinned by differences in sensitivity to insulin/TOR. In *D. melanogaster* the wing disc shows high sensitivity to insulin/TOR, whereas the genital disc shows low sensitivity [126]. In genital discs, low sensitivity to nutrition is achieved by modifying the expression of a negative regulator of the insulin signaling pathway, Forkhead BoxO (FoxO). In *D. melanogaster*, the genital discs become insensitive to insulin/TOR signaling by expressing very low concentrations of FoxO protein [127], while *O. nigriventris* genital discs achieve the same effect by expressing high levels of FoxO protein [128]. In both cases, these changes in FoxO concentration ensure that insulin signaling in genital disc cells remains more or less constant across a range of nutritional conditions [126]. Hyperallometric traits, such as the horns on male rhinoceros beetles, typically are show increased sensitivity to insulin/TOR signaling [129]. At least in this beetle, this is due to differences in the levels of InR in these tissues [129]. Thus, allometric coefficients differ between organs at least in part due to differences in sensitivity in insulin/TOR signaling [3].

In addition to the rates of growth, the relative timing of the growth period controls body size by modifying the duration of the growth period in insects and other animals [38, 130–133]. Growth duration is regulated by the production and secretion of hormones important for setting the pace of development, and is also controlled by the insulin/TOR signaling. In particular, in insects insulin/TOR signalling regulates the synthesis of the steroid hormone ecdysone, the hormone responsible for regulating the time of moulting across all larval stages and finally metamorphosis in all holometabolous insects [134, 135].

Ecdysone is synthesized from cholesterol in the prothoracic gland (PG) of insects and released into the circulating hemolymph [136]. In the fat body, it is then modified to its active hydroxylated form, 20-hydroxyecdysone (20E), by a P450 monooxygenase [135]. 20E binds to a nuclear receptor formed from a heterodimerization of Ecdysone Receptor (EcR) and Ultraspiracle (Usp) [137, 138], this receptor binding activates stage-specific cascades of gene expression that determine the timing of developmental processes [139].

In the final larval instar, a series of three smaller pulses of ecdysone prepares the animal for metamorphosis before the final pulse induces the onset of pupal development [140]. The first of these smaller pulses is sensitive to nutritional conditions, and induces a developmental transition known as critical weight [66, 132, 140, 141]. Critical weight defines a developmental transition in the way larvae respond when starved. Larvae starved before critical weight delay initiating metamorphosis for up to ten days, after critical weight larvae no longer delay the onset of metamorphosis when starved [133, 141–143].

Insulin/TOR signaling regulates when larvae reach critical weight [38, 130–133]. It does this by acting on the PG to regulate the timing of first pulse of ecdysone synthesis [144], at least in part by regulating the ploidy of PG nuclei [145]. By regulating when larvae initiate metamorphosis, the ecdysone pulse at critical weight determines for how long organs can grow.

These small pulses of ecdysone also act to modulate organ growth rate [66, 146]. The wing imaginal discs [66, 147], ovary [148, 149], and the medulla neuroblasts in the central brain [150] all depend on ecdysone for growth. This dependency is important for whole organ size, but also for coordinating growth across compartments within an organ. Ecdysone signals regulate the growth of both anterior and posterior compartments of the *Drosophila* wing to produce appropriately proportioned wings [147, 151]. This highlights the role of ecdysone in regulating organ shape.

While the amount of time an organ has to grow clearly impacts its final size, the relative timing of when organs initiate growth, a phenomenon known as heterochrony, also influences body and organ shape [152]. Changes in the onset, duration, and rate of growth underlie major divergences in skull morphology in mammals and their close relatives [153]. In particular, accelerated ossification of the bones of the cranial vault in mammals as compared to non-mammalian amniotes can describe their relative brain sizes [154]. While it is unclear what leads to differences in relative timing of bone ossification among taxa, variation in the timing of ossification

correlates with the developmental origin of the bone – where the timing of ossification occurs earlier in dermal bones, and much later in endochondral bones [154]. This suggests that the timing of ossification is independently regulated between bone types, and further highlights an important manner in which developmental programs can impart modularity to body and organ shapes.

Finally, a number of recent studies have demonstrated that in addition to their role in organ autonomous size regulation, morphogens can be secreted into the circulatory system to regulate body and organ growth. In *Caenorhabditis elegans,* morphogens like DBL-1 (orthologous to Dpp) are secreted from several classes of neurons and act systemically to regulate body size and male tail shape [155–157]. DBL-1 plays important roles in metabolism and lipid storage, and acts upstream of insulin signalling to exert these effects [158] . Similarly, in *D. melanogaster* morphogens like Hedgehog and Activin-β are secreted by cells of the midgut and act remotely to regulate peptide signals from the fat body, ILP secretion, and ecdysone synthesis [159–161]. While enteroendocrine cells in the midgut increase Activin-β secretion when larvae are fed high sucrose [161], the enterocytes of the midgut increase Hedgehog secretion when larvae are starved [160]. Interestingly, secreted systemic Hedgehog does not modify the Hedgehog morphogen gradient in the wing imaginal disc [160]. Thus, in addition to their roles controlling local organ growth patterns, morphogens can act systemically to cue changes in hormone production and secretion to regulate whole body growth.

Mechanisms regulating organ shape

The role of Morphogens in defining cell identity and behaviour In addition to regulating organ growth, morphogens designate spatially structured patterns across a field of cells. Graded morphogen signals determine the position, arrangement, and fate of cells depending on the concentration each cell receives. Bicoid is the first identified and most broadly studied morphogen [162–164]. *bicoid* mRNA is loaded into the anterior pole of the *D. melanogaster* egg during egg development [165]. Its translation during embryogenesis results in an anterior to posterior gradient of protein. This gradient specifies the antero-posterior axis of the embryo and in setting off the cascade of signalling interactions that establish segments along the anterior/posterior axis of the embryo [162]. Similarly, the Dpp gradient in the *D. melanogaster* wing regulates growth and establishes boundaries of gene expression that are essential for the correct specification and positioning of the veins along the wing [166]. In this way, morphogens not only regulate the relative size of organs, they also regulate organ shape by controlling the identity, position, and behaviour of cell types within an organ.

Morphogens do not function only in animals, but play important roles in establishing cell identity in plants as well. Plant plasma membranes are bound by rigid cell walls that separate one cell from another [167, 168]. However, vascular networks connect cells and tissues, allowing communication between them [169, 170]. Plant morphogens called auxins are produced in immature shoots and travel to the roots and apical parts of the plant through these vascular networks [171]. Within the plasma membrane of individual plant cells, localized efflux proteins (auxin transporting cells) convey auxin in and out of the cell. Studies into the auxin families show that the membrane localized PIN FORMED (PIN) proteins are involved in instructing auxins on the direction and rate of their travel [172]. PIN1, a subclass of the PIN proteins, is involved in patterning the venous network such that new veins are connected to older ones as well as guiding the formation of veins into target tissues and consequently determining the site for a new organ initiation [173, 174].

Via their role in patterning, morphogens tell cells how they should behave. These cell behaviours define organ shape by: (i) changing cell shape as a result of cell identity, (ii) inducing cells to proliferate and/or enlarge in size, (iii) causing cells to migrate or reorganize within a tissue, or (iv) triggering cell death which can be seen as a cessation of growth and/or loss of cells [175] (Fig. 3).

How cell behaviour shapes organs In the simplest scenario, organ shape is a direct function of the shapes of its constituent cells [181–183]. For example, epithelial cells are cuboidal, while neurons have more radiating shapes with obvious projections [184] (Fig. 3). Differences in cell shapes arise through mechanical reorganization of their cytoskeleton [185]. For example, in neurons the microtubule cytoskeleton organizes itself to form highly ordered bipolar spindles [186]. In this way, the shapes of specialised cell types dictate the range of potential shapes of an organ.

Even amongst cells of the same type, cell shape plays an important role in organ shape. When the sepals of *Arabadopsis thaliana* flowers develop, the cells in the meristem show variable growth rates among cells. Growth rates among cells differ due to spatial and temporal variation in cell stiffness [187]. This spatial and temporal variation is regulated, in a process called spatiotemporal averaging. Disruption of spatiotemporal averaging, such as found in plants mutant for *FtsH4*, makes growth rates more uniform [187]. This creates greater variability in cell size and shape – resulting in misshaped sepals [187]. Thus, organ shape relies on the

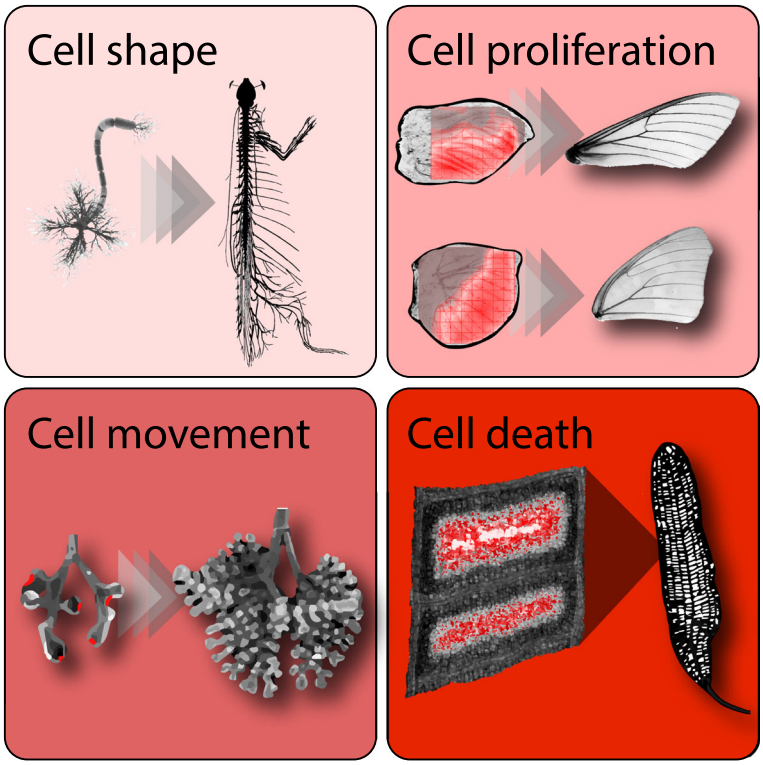

Fig. 3 Organ shape is generated by four types of cell behaviours: cell shape, cell proliferation, cell movement, and cell death. Cell shape: The elongate shape of neurons allows the central nervous system to convey information throughout the whole body. Cell proliferation: differing patterns of cell proliferation (cells in red) in the developing wings of the moth *Manduca sexta* and the butterfly *Junonia coenia* generate elongate versus triangular wings in the adults [176]. Cell movement: Invaginations are caused by apical constrictions in the lung epithelium (cells in red), and are responsible for producing the branching patterns of the lung tissue [177, 178]. Cell death: Programmed cell death in cells between the lateral and longitudinal veins in the leaves (cells in red) give the lace plant its lattice-like leaf shape [179, 180]

ability of organs to dynamically modulate cell behaviours contributing to the shape of their constituent cells.

Cell division can also play an important role in regulating final cell shape. The morphogens that instruct differences in cell identity also regulate the direction and rates of cell division across fields of cells [188]. In the wing of *Drosophila,* the orientation of cell divisions determines the shape of the tissue fields between wing veins [181]. Narrow intervein regions arise due to cell divisions that are oriented perpendicular to the long axis, whereas broader intervein regions show more random orientations of their cell divisions [181]. Conversely, the wing shapes differ between the moth *Manduca sexta* and the butterfly *Junonia coenia* due to different patterns of localised cell proliferation [176] (Fig. 3). Wings in *Junonia coenia* are triangular and show more even distributions of proliferating cells within the distal regions of the wings. *Manduca sexta* more elongate wing arises because patterns of proliferation are shifted towards the posterior portion of the wing [176]. Thus, the way cells proliferate, by dividing along oriented planes or via localized patterns of cell division acts to shape organs [189].

Organ shape is also affected when cells rearrange. Shape formation from cell polarity during tissue rearrangement can result from narrowing and lengthening (convergence and extension respectively) of rows of cells during development, where cells intercalate between other cells to achieve this process. Convergence results in a narrowing of tissues in a mediolateral direction while extension elongates tissue from head to toe [177, 190]. In amphibians, convergence and extension shape the notochordal and somatic tissues, while in other vertebrates these processes shape the notochord, dorsal axial, and paraxial mesodermal tissues [178]. Both acting to regulate cell activities and enhance tissue shape formation.

Invagination and cleft formation provide additional cases of how cell rearrangement is important for reorganizing simple cells into complex, branched, and multilayered structures. In the salivary and mammary glands, digits, lungs (Fig. 3), and kidneys, epithelial cells form branched, tree-like structures [177, 191]. This branching is produced by invagination and cleft or bud formation in cells. During invagination, the apical region of cells becomes constricted and causes the cells in this

region to become wedged shaped, inducing the cells to bud off and protrude [184, 192]. The cleft formation then generates new tips from the pre-existing branch, splitting it into two or three tips. This is also observed in plants where the shoot and floral meristem branches off to form differing numbers of petals, stamens, carpels, and sepals and more visibly in the branching patterns of leaf venation [169, 193]. This process in both plant and animals regulates cell behaviour and contributes to produce diversity in organ shapes based on the location of cells and their subsequent functions.

Finally, cell death plays vital roles in the shape of many organs. Perhaps most famously, cell death defines the shape of the vertebrate forelimb. Cells in the interdigital regions die during embryogenesis in many vertebrates, giving rise to the separated digits of the hands and feet [194–197]. The extent to which cells die in this region determines whether the limb will have separated digits, like in the chicken [194], or webbed wings, as in bats [198]. In both cases, cell death is initiated in response to signals from Bone Morphogenetic Proteins (BMP), specifically BMP2, BMP4, and BMP7 [199]. Similarly, programmed cell death in plants generates a range of leaf shapes, including lobed or lattice-like leaf shapes [200–204]. The leaves of the lace plant acquire their characteristic, intricately fenestrated leaf shape via punctuated patterns of cell death in the cells found between longitudinal and transverse leaf veins [179] (Fig. 3). These patterns are induced in response to the phytohormone ethylene, which is produced by the leaf cells and stimulates death in the intervein cells [180, 205]. In both cases, to achieve correct organ shape cell death is induced in response to signals from morphogens or hormones.

As cells adopt their instructed behaviour, mechanical forces generated during different events like cell division, growth, movement, and cell death contribute to determining the final shape of the organ. These forces can act at the level of an individual cell or across a whole tissue [206]. They are generated by the molecular components that provide cell structure and can arise from either intrinsic or extrinsic factors. Intrinsic forces control the movement of the cytoskeleton during cell division and cell differentiation [207]. Extrinsic forces regulate cell-to-cell interactions, and cell-extracellular matrix interactions during cell processing and repackaging – known as tensile force [208, 209]. In many cases, like in the human lung, final organ shape is a result of both intrinsic and extrinsic forces. Here, the shape of the lungs arises from the apical constriction of lung cells and from cell-cell adhesion to neighbouring cells and the extracellular matrix [210]. In this way, the cell identities acquired through morphogen activity along with the mechanical properties of the tissue itself are important in defining the shape of the organ.

Finding the common ground – body shape and organ shape

As biologists have long been interested in variation in body size, body shape, and organ shape, they have uncovered a multitude of examples where size and shape co-vary [14–16, 32, 33, 64, 211]. While throughout this review we have separated our discussion into either the developmental mechanisms that regulate body shape or those that regulate organ shape, it is clear that in many cases these mechanisms must overlap. New genetics tools, which allow researchers to measure quantitative variation in signalling pathways in real time, have the potential to shed important insights into how and when the developmental mechanisms regulating body and organ shape are shared.

Drosophila wings provide a clear example of overlapping mechanisms regulating relative size and shape. Wings decrease in size with increasing temperatures in *D. melanogaster* and its close relative *D. simulans* [32, 60]. In *D. simulans,* wing shape also changes with temperature. Approximately 20% of the shape changes in the wing correlate with wing size, suggesting that some of the variation in wing shape might share common regulatory mechanisms with those that create variation in wing size [60].

Wing shape is commonly characterised using the relative positions of the veins [60, 212, 213]. The morphogen pathways, like Dpp, Epidermal Growth Factor, and Hedgehog, that regulate wing growth also set up the position of the longitudinal wing veins in the third instar larvae [166, 214]. Hedgehog and Dpp in particular establish the positioning of the longitudinal veins in the growing disc [214]. These longitudinal veins are responsible for most of the shape changes in temperature that correlate with wing size [60]. Variation in morphogen signalling in response to temperature during larval stages potentially affects both the relative size of the wing and the position of veins, thereby contributing to both body and organ shape.

Of course, we would not expect the mechanisms regulating organ shape to always be shared with those that regulate body shape. Instead, they would overlap only when organs are both growing and patterning. For example, later in pupal development the *Drosophila* wing undergoes only minimal growth. During this stage, morphogens like Dpp act to refine the position and differentiation of the longitudinal veins. Variation in Dpp signalling in response to temperature in the pupal stages would be likely to generate variation in wing shape that is uncorrelated with wing size. Explicit experiments manipulating the activity of these morphogens at defined

intervals of development would help to explain when body shape and organ shape share the same genetic underpinnings. More importantly, this example illustrates that knowing when growth and patterning occur in developmental time will help to inform whether the extent to which body and organ shape should co-vary.

While we know a considerable amount about the regulation of body and organ shape in *D. melanogaster* and other insects, uncovering the genetic underpinnings of co-variation in body and organ shape need not be limited to model organisms. Careful examination of changes in organ size and shape over developmental time would provide a simple manner to understand how each process is regulated, and can also offer insights into the types of mechanisms at play. These studies could identify relevant pathways for study. Further with the advent of CRISPR, mutations can be introduced into most genes for many organisms. This allows researchers not only to explore the effects of loss-of-function for candidate genes that regulate organ size and shape, but also permits the introduction of fluorescently-labelled proteins into their endogenous location – facilitating quantitative studies of protein concentration.

Detailed quantifications of each of the relevant pathways that regulate growth and patterning over developmental time is likely to provide the deepest insights into how body and organ shape are co-regulated. Having said this, multiplexed quantification of signalling pathways is challenging even in well-studied model organisms. One way of simplifying this task is to formulate theoretical models that will predict how morphogens and systemic signals intersect both temporally and spatially to generate the appropriate body and organ shape. At their best, these mathematical models would generate precise hypotheses with regards to how body and organ shape are regulated, which can be subsequently tested with experimental methods. These types cross-disciplinary approaches, between those interested in variation in morphology, developmental biology, and systems biology, will enhance our ability to uncover the genetic mechanisms regulating body and organ growth for a broader range of organisms.

Finally, while the evolution of body and organ shape among taxa generates an impressive array of morphological diversity, how the genetic mechanisms that regulate body size and shape change remains an open question. It is clear that the extent to which organs change their shape depends on their function. Myriad examples of exaggerated sexually dimorphic traits illustrate how organ form can vary greatly when under sexual selection. In contrast, for organs that perform multiple functions, changes in size and shape might be strongly canalised to avoid trade-offs. Future studies comparing the how size and shape mechanisms evolve will help to elucidate how the diversity of body and organ shapes have arisen.

Conclusions

With recent insights into the developmental mechanisms that control growth, our understanding and some of the basic principles that govern growth processes has significantly expanded. However, there is still much left unknown. In this review, we identified the different approaches used to study body shape, using the relative size of organs, and those that describe organ shape. We hypothesize that in some cases the mechanisms that regulate organ shape could overlap with those that regulate organ size. It is our hope that these ideas will fuel further research into exploring the mechanisms regulating the vast diversity of body and organ shapes.

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AC and CM contributed equally to the developing the ideas and writing this paper.

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